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THE TOXICOLOGY OF DMMPA. PART I. THE ACUTE EFFECTS OF DMMPA,(U)
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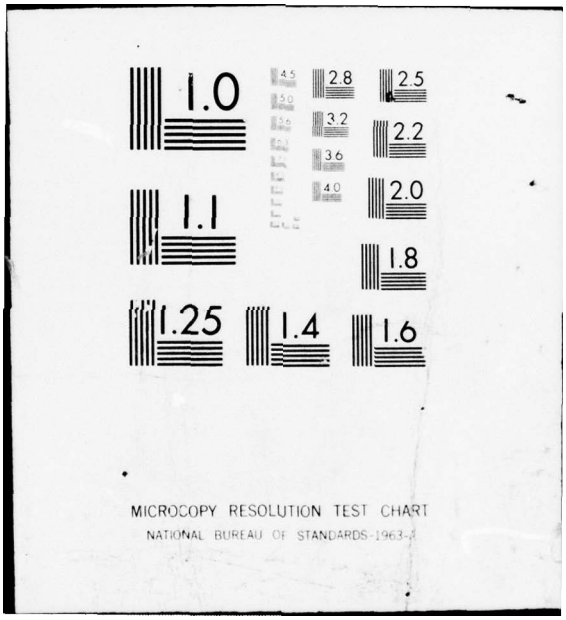
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SUFFIELD TECHNICAL PAPER

NO. 466

TOXICOLOGY OF DMMPA

PART I

THE ACUTE EFFECTS OF DMMPA

by

I.W. Coleman

PROJECT NO. 13E11

August 1977

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

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SUFFIELD TECHNICAL PAPER NO. -466

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(12) 56 p.

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ACKNOWLEDGEMENTS

The author wishes to gratefully acknowledge the assistance given him in this study by Mr. H.T. Copeman for physiological measurements, Mr. L. Lemna for haematology and biochemical estimations, Mr. W. Blades for supervision of animal arrangements and Mr. J.H. Bailey, Mrs. E.E. Sweet, Mr. W. Engel and Mr. R.R.F. Newnham for expert animal handling. Thanks also are due to Mrs. W.D. McNally for serum determinations of DMMPA.

TABLE OF CONTENTS

FOREWORD

In conducting the experiments described in this report, the author and his staff have adhered to the code of ethics and the methods promoted by the Canadian Council on Animal Care. These are contained in their bulletin "Principles of Care of Experimental Animals: a Guide for Canadian Users".

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
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THE TOXICOLOGY OF DMMPA
PART I
THE ACUTE EFFECTS OF DMMPA (U)

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ABSTRACT

The toxicological properties of dimethyl morpholinophosphoramidate (DMMPA) have been examined in mice, rats and rabbits using a variety of criteria of toxicological responses. Nutritional, physiological, haematological and blood serum components were studied after DMMPA acute dosing. Macroscopic and microscopic pathology was also carried out. The maximum dose causing no pathological effects was in no case less than the LD_0 dose estimate, indicating the compound was remarkably free of pathological effect, and likely indicating the compound to be safe for acute exposures of small amounts to man.

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DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
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THE TOXICOLOGY OF DMMPA
PART I
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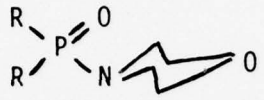
INTRODUCTION

A compound approximating the physical properties of an organophosphorus agent, but lacking the extremely high lethality, can have many applications in the testing of protective systems against the nerve agents. It can be personal decontaminants, modes of operation within a mimic toxic environment as well as safe studies of disposal devices and procedures which do not involve environmental contamination with highly toxic materials. To meet such requirements, the compound must be a colourless liquid of low odour having a density and viscosity near that of a possible agent (e.g. VX); be in range of the vapour pressure of a possible agent; be absorbed by skin and other tissues and be relatively stable in metabolism after absorption and excreted, if not quantitatively, at least reproducibly. Above all, it must demonstrate a low toxicity such that it can be safely used in human trials where assessment of the effectiveness of protective devices and procedures can be made without assumptions. The compound must also lend itself to analysis in blood and urine at high sensitivity and specificity without interference from other blood or urine constituents, whether normal or the result of ingestion of common drugs such as aspirin or alcohol, varieties of food flavours or the result of habits such as smoking.

To meet such a need Adie et al. (1) examined a large number of compounds and tested the usefulness of benzyl salicylate in such a capacity, but found it inadequate in certain aspects. Attention was directed to compounds of the morpholino-phosphoramide type and Jardine et al. (2) prepared



the R = C_2H_5 derivative. Diethyl-4-morpholinophosphoramidate (DEMPA) was subjected to a variety of analytical and animal studies and found in many aspects to be acceptable. However, in batch production of DEMPA by standardization procedures some batches were found to have a toxicity 20-30 times that of others, increasing the toxicity of the product to unacceptable levels. Simple and direct procedures to purify such batches failed and the compound was abandoned in favour of the dimethyl derivative (3) which in laboratory batch production produced a reasonably uniform low toxic product even less toxic than the diethyl compound. Preliminary studies of the analytical properties as well as toxicity in rodents (4) were so highly favourable that a detailed study of the safety of the compound was initiated.

SECTION A

ACUTE LETHALITY OF DMMPA

Dimethyl-4-morpholinophosphoramidate (DMMPA) is a clear, faintly yellow liquid having an odour reminiscent of choline. It has a vapour pressure of approximately 10^{-2} mm Hg; a density of 1.223 (20°C) and a refractive index of 1.4530 $D^{25\cdot2°C}$. It is highly water soluble.

In Part I of this report, the effects of acute single doses of DMMPA to animals will be reported, with the effects of chronic dosing of DMMPA in rats reported in Part II.

The LD_{50} values of DMMPA were determined in albino mice, an inbred strain obtained from University of Alberta Vivarium, Edmonton; albino rats were purchased from High Oaks Farms, Ontario and New Zealand white rabbits from Jansen Enterprises, Calgary, Alberta. With the exception of the rabbit study where only 6 animals per dose were used, dose group size was maintained at 10 per group. Assays were considered acceptable only if a minimum of 4 partial kill groups (usually five) were obtained as well as zero and 100% kills. DMMPA was administered neat in all determinations with the exception of the intravenous determination in mouse and in the tests on new-born rats, where the material was diluted with water to give measurably accurate microliter volumes when dispersed by either Agla or Hamilton syringes. All animals tested were healthy young adults retained in the animal quarters for at least one week after shipment before use. The new-born rat pups were selected from breeding of the same supply stock "in-house" with unsexed pups 1-3 days old used in the assessment. All assessments of mortality were made after 24 hours and LD_{50} parameters calculated by probit analysis using a computer and by the graphic procedure of Litchfield and Wilcoxon (6).

Results

The LD_{50} values for DMMPA are shown in Table I. The intravenous toxicity in mice after tail vein injection was 422 mg/kg. There was no evidence of slough at the site of injection. Animals died within 1-2 hours with no delayed deaths. Toxic signs were minimal with the mice showing a period of hyperactivity immediately after injection followed by prostration and difficulty in breathing. Animals died from anoxia. There was a low incidence of convulsions. In the rabbit where DMMPA was applied via ear vein there was again no evidence of local reaction or sloughing. The approximate LD_{50} of 347 mg/kg agrees with that found in the mouse. Toxic signs in the rabbit were also meagre. There was no hyperactivity shown in this species, merely a gradual paralysis coupled with gasping respiration leading to cyanosis and death by respiratory failure. Times to death in the case of the rabbit were somewhat prolonged although no deaths occurred longer than 5 hours after dosing. DMMPA is about 1/10 as toxic by the intramuscular route in the mouse

TABLE I

ACUTE TOXICITY OF DMMPA

Route of Application	Species	Weight Range (g)	Animals per dose group	LD_{50} (mg/kg) (g)	Range for 95% Confidence	Slope Function
Intravenous	Mouse (M)	26-28	10	422	396-442	10.5
	Rabbit (M)	2500-3500	6	347	---	---
Intramuscular	Mouse (M)	26-28	10	4819	4366-5296	4.71
	Rat (M)	175-190	10	5200	4815-5620	5.43
Intraperitoneal	Mouse (F)	24-26	10	5200	4860-5510	6.28
	Mouse (M)	26-28	10	5480	5130-6000	5.29
	Rat (Adult M)	175-190	10	2370	2210-2530	6.38
	Rat (Newborn)	4-7	10	6760	4070-8070	1.12
Oral	Rat (M)	175-190	10	5910	5380-6570	4.91
	Rat (F)	155-170	10	6510	5790-7330	3.17
	Rat (Fasted M)	175-190	10	5840	4860-6600	4.55

in comparison with the intravenous route. The value of 4819 mg/kg in the mouse by intramuscular application is near that found in the rat for the same route. However, in the rat the toxic signs are somewhat different in as much as there is evidence of excessive salivation and bloody tears in this species not seen in the other two. There were also signs of rear limb weakness leading to paralysis. The rat death was, however, similar to that of the other species, namely respiratory failure.

The intraperitoneal LD_{50} of DMMPA in the mouse was about 5000 mg/kg. From Table I it can be seen that there is no significant difference in this toxicity in males or females. The intraperitoneal LD_{50} of DMMPA in the adult rat is about double that found in the mouse. However, 1-3 day - old new-born rat pups were much more resistant to DMMPA than the adult. Oral administration of DMMPA to rats using a ball-tipped needle indicated an LD_{50} around 6000 mg/kg. Again in this species males and females were shown to be equally sensitive. The oral LD_{50} was not changed in rats deprived of food (but not water) for 24 hours.

SECTION B

ACUTE PHYSIOLOGICAL EFFECTS OF DMMPA

New Zealand white rabbits of both sexes were anaesthetized with urethane (6.0 ml/kg of 25% urethane in water). The carotid artery was cannulated and blood pressure recorded via a Statham transducer; the jugular vein was cannulated for infusion; trachea cannulated for respiration rate and volume recording and electrodes attached to the trachea and inside rectum for electrocardiograph leads leading to a heart rate counter. All parameters were recorded on a Sanborn recorder which also carried a dose marker and time interval record.

In order to delineate the sequence of events, both continuous infusion and time separated dose applications were used giving similar results. However, dose separation seemed to be more useful in demonstrating the effects.

Results

Typical responses of the rabbits are exemplified in Figure 1 in which the percentage change in the parameters with successive doses of 200 mg/kg of DMMPA are plotted: all values shown are relative to the initial predose levels of the four parameters.

Respiration increased in both rate and volume after the initial dose of DMMPA but declined on successive doses to near or slightly above the predose levels. Heart rate decreased with successive doses of DMMPA to a value about 30% of normal after 5 doses. Blood pressure declined from the first dose application, falling after successive doses so that the blood pressure had fallen to 50% of control levels after 5 successive doses.

Although the dosage applied in separate doses in the above experiment was 3 times the acute instantaneous dose (330 mg/kg), the animals did not die, but continued to improve to normal values after 2 hours when the experiment was terminated. Figure 2 describes an experiment in which the above was duplicated, except that doses of DMMPA at 200 mg/kg were repeated until the animal died. As with the studies illustrated in Figure 1, the cardiovascular system, as represented by heart rate and blood pressure data, was demonstrably the more sensitive, declining to 30% and 20% respectively of control values after 17 doses. Respiration rate under these conditions showned a steady decline but after 17 doses still retained 65% of normal pre-dose levels. Respiration volume showed a compensatory increase with successive doses reaching peak compensation after 8 doses but declining steadily thereafter to normal volume just before death.

From the above data, the prime factor in death from poisoning with DMMPA is cardiovascular, even though the most obvious sign after acute lethal application of DMMPA is anoxia. This concept is supported by the findings of acute haemorrhage and congestion in the lungs, and occasionally in the liver and kidney, found on autopsy and verified by histological section (see pathological effects of acute DMMPA dosage).

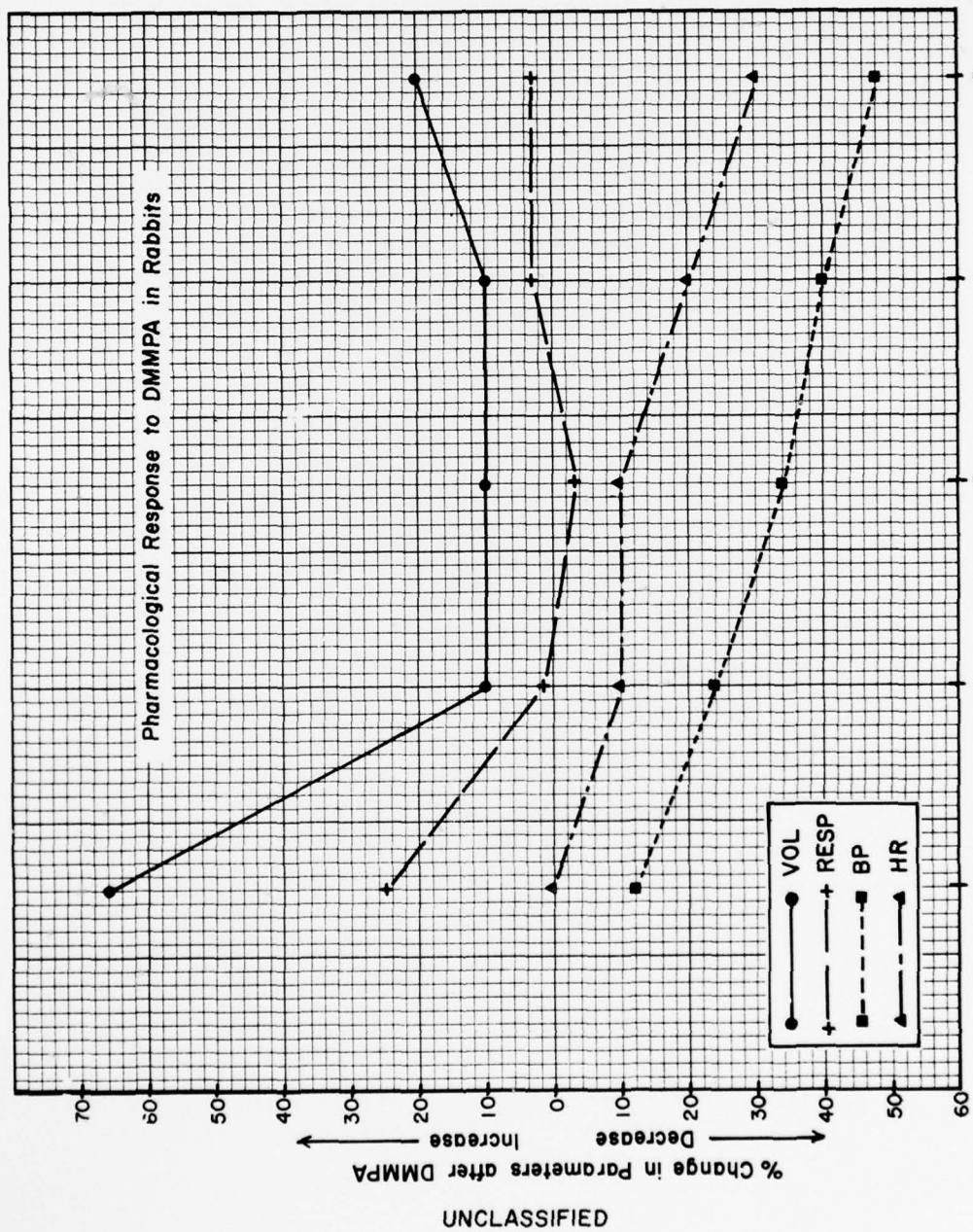


FIGURE 1: RESPIRATION RATE AND VOLUME, BLOOD PRESSURE AND HEART RATE IN RABBIT ANAESTHETIZED WITH URETHANE AFTER 5 INJECTIONS OF DMMPA AT THE LEVEL OF 200 MG/KG EACH DOSE, THIS ANIMAL SURVIVED, PARAMETERS ARE PLOTTED AS PERCENT CHANGE OF PREDOSE LEVELS.

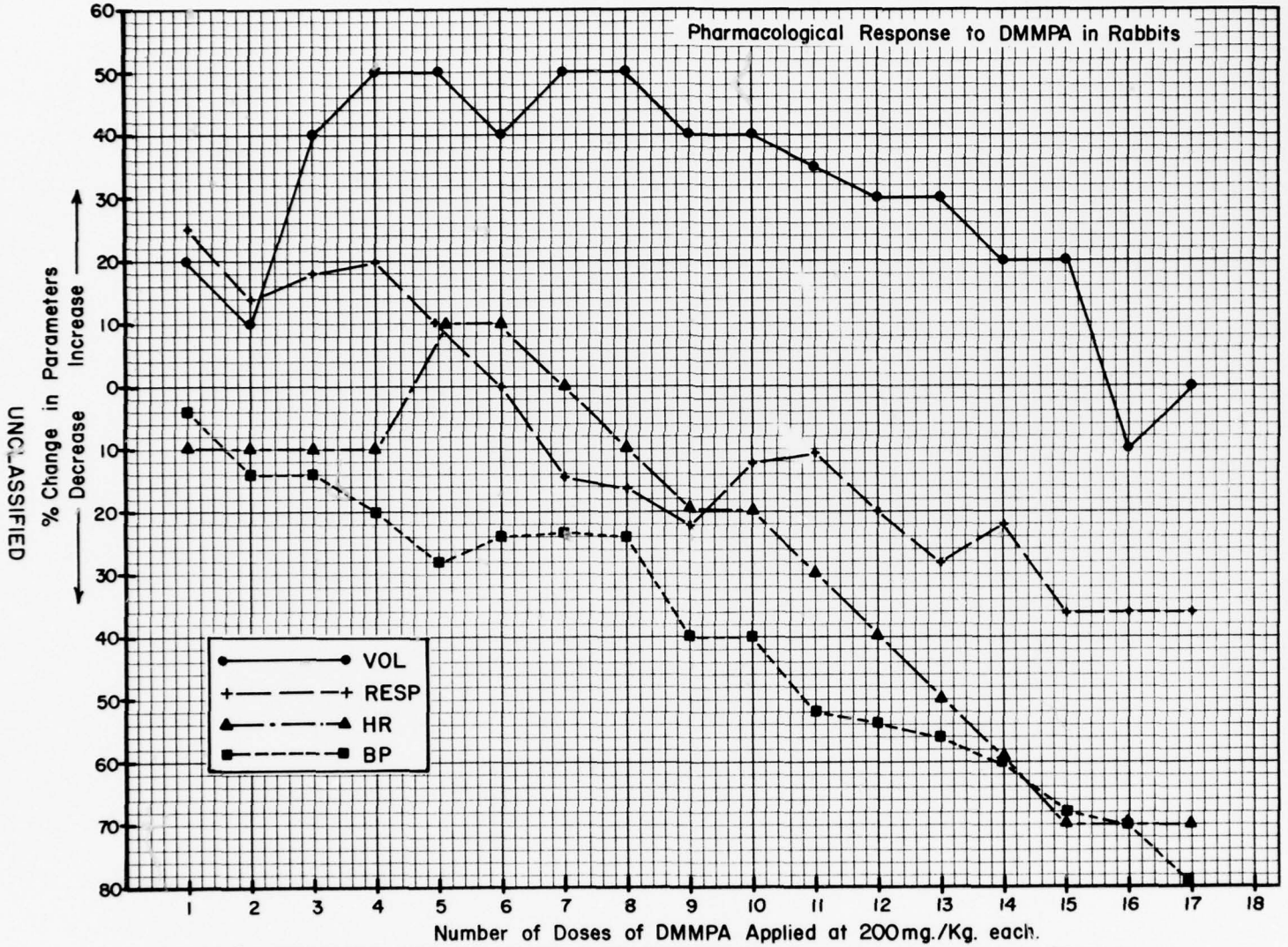


FIGURE 2: RESPIRATION RATE AND VOLUME, BLOOD PRESSURE AND HEART RATE IN RABBIT ANAESTHETIZED WITH URETHANE AFTER 17 SUCCESSIVE INJECTION OF DMMPA AT 200 MG/KG EACH DOSE. THIS ANIMAL DIED 2 MINUTES AFTER THE LAST DOSE OF DMMPA. PARAMETERS ARE PLOTTED AS PERCENT CHANGE OF PREDOSE LEVELS.

Clearance of DMMPA from Rabbit Blood

New Zealand white rabbits of both sexes were anaesthetized with urethane and the femoral vein was cannulated with polyethylene tubing left in situ. Doses of DMMPA from 5 to 50 mg/kg were administered and washed into the animal with saline. Blood samples of 2.0 ml were withdrawn at regular time intervals and this blood was analysed for DMMPA after extraction with chloroform using the gas liquid chromatographic procedure of McNally and Adie (3).

Results

The blood levels of DMMPA in rabbits after dosing with 5, 10 and 50 mg/kg are given in Table II. These data were converted to the percentage of the peak value and the logarithm of these values plotted against time. The best straight line was fitted to these data from which the time required to reduce DMMPA blood levels to 50% of peak value was read as the '$t_{\frac{1}{2}}$' value. These data gave the $t_{\frac{1}{2}}$ for DMMPA in rabbits to be 43 minutes.

SECTION C

ACUTE OPHTHALMIC EFFECTS OF DMMPA

(a) General Eye Damage

Two groups of six New Zealand white rabbits (2.5 to 3.5 kg) were lightly restrained and exposed to 40 and 100 microliters of neat DMMPA respectively. The material was dropped directly on the corneal surface. The animals were individually caged and examined after 5, 24, 48, 72 and 96 hours. Damage in the external eye-lid, conjunctiva, cornea and sclera was scored according to the scheme outlined in Annex A. Total damage score was represented by the sum of the individual sub-scores to give a subjective assessment of the extent of damage. All animals were examined by the same observer (I.W.C.) so that bias would be uniform over the entire test.

TABLE II

LEVELS OF DMMPA IN RABBIT BLOOD WITH TIME

Time After Dosing (minutes)	DMMPA - Micrograms per ml blood						
	5 mg/kg			10 mg/kg		50 mg/kg	
	1	2	3	1	2	1	2
0.5	41.1	16.8	---	32.0	---	92.5	110.0
2.0	29.5	15.2	25.0	25.0	---	172.0	206.0
4.0	18.8	13.3	19.3	16.8	21.7	75.0	145.0
8.0	18.1	13.8	18.5	14.5	20.4	70.0	153.0
16.0	17.8	14.4	17.8	14.3	20.6	67.0	129.0
32.0	10.5	9.8	14.0	9.3	9.0	50.0	125.0
60.0	8.9	9.4	10.0	9.7	9.4	40.0	101.0
90.0	7.8	10.3	---	7.3	4.7	35.3	71.0
120.0	7.8	6.8	---	6.8	---	28.8	68.0
180.0	4.9	3.8	---	4.2	---	21.0	20.0

Results

All animals showed a forceful withdrawal action when DMMPA was applied indicating that the compound caused immediate eye irritation of some intensity possibly similar to many common solvents. If the reaction was painful, it was not of sufficient intensity to induce vocalization.

Of the eye structures, the conjunctiva was most severely affected, with most animals (after 5 hours) demonstrating an erythema with swelling with evidence of sloughing. The nictitating membrane was next most seriously affected followed by similar scleral damage. The cornea was not damaged. The mean damage scores for the two groups receiving 40 and 100 microliters of DMMPA are plotted against time in Figure 3. As can be seen, maximum damage was observed at 5 hours with all animals demonstrating almost complete recovery in 24 hours. However, 96 hours of recovery time were required before the scores returned to normal values.

Effect of DMMPA on Intraocular Pressure

Two groups of six New Zealand white rabbits were lightly restrained and exposed to 40 and 100 microliters of DMMPA by direct deposit in the right eye. Measurements of intraocular pressure by Schotz Tonometer were made prior to the test and 24, 48 and 72 hours after the test.* Each measurement was the average of four observations.

Results

The averages of the observed intraocular pressure for both groups are given in Table III. From these observations it was concluded that DMMPA showed little lasting effect on the intraocular pressure. However, this conclusion is limited in that variation in the individual animal with time was found to be substantial and not shown in the average values given in Table III. Much of this variation is due to the method of measurement.

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* Note: This test was performed on different rabbits from those used for the eye damage test. It was found that the four measurements made each time and average produced a mild irritation on the rabbit eye that interfered with the irritation due to DMMPA alone.

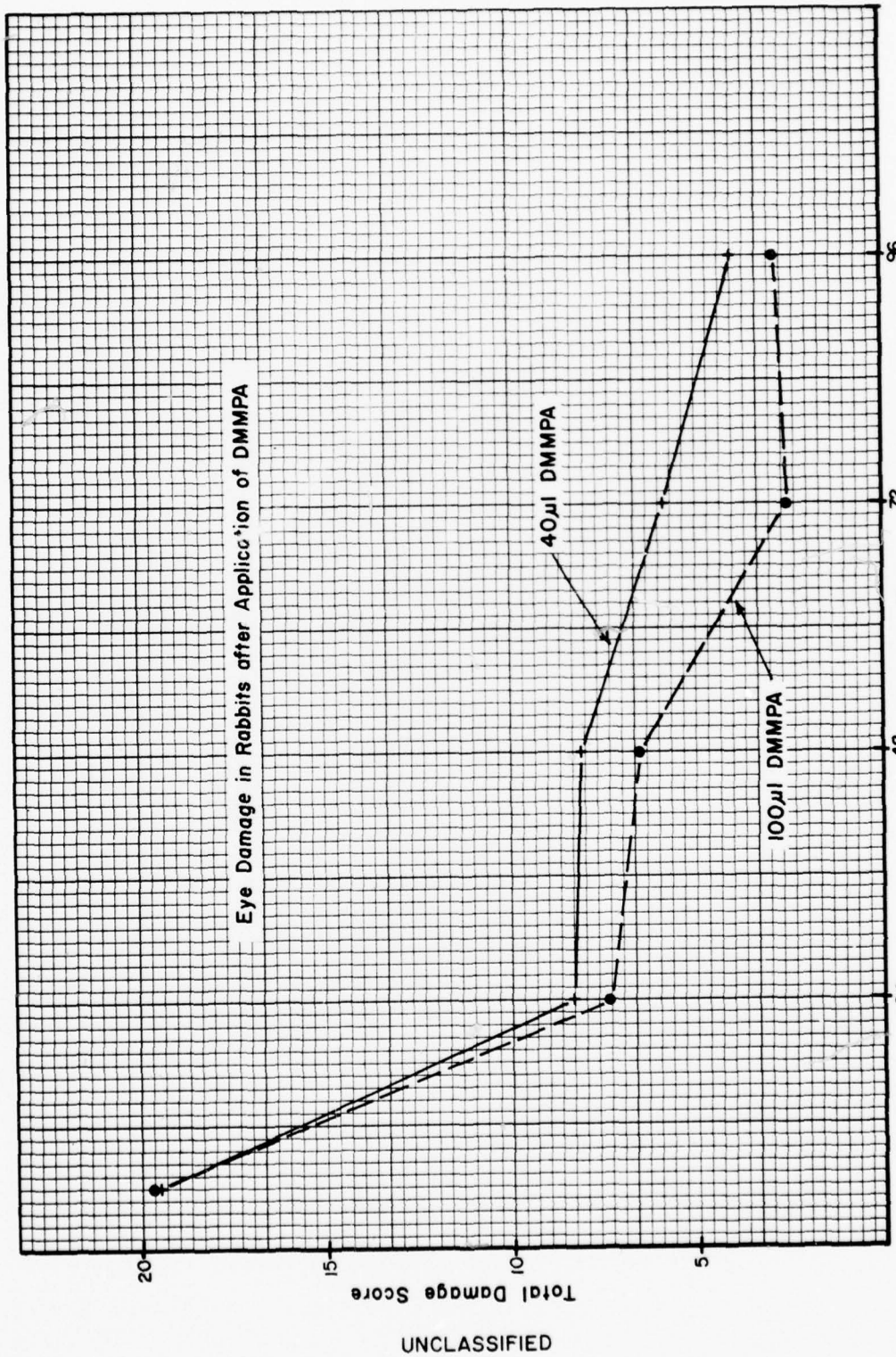

FIGURE 3: TOTAL AVERAGE DAMAGE IN EYES OF A GROUP OF 6 RABBITS WHERE 40 MICROLITERS OR 100 MICROLITERS OF DMMPA HAS BEEN INSTILLED INTO THE RIGHT EYE OF THE UNANAESTHETIZED ANIMAL.

TABLE III

INTRAOCULAR PRESSURE AFTER DMMPA IN RABBITS

DMMPA (micro-liters)	Intraocular Pressure in mm Mercury *			
	Prior to Application	24 hrs	48 hrs	72 hrs
40	14.6	17.0	13.8	14.2
100	14.0	15.7	11.8	15.4

* Each value represents the mean of 6 animals with four observations made for each individual determination.

SECTION D

TOXIC EFFECTS OF DMMPA AFTER ACUTE SINGLE DOSE APPLICATION

Eight groups of 15 female rats were stabilized in metabolism cages for one week prior to the test. The animals were weighed and distributed into 8 groups having a mean weight of 175 grams each and insignificantly different in weight distribution one from another. Six groups were dosed with DMMPA neat in volumes beginning at 0.1 ml per animal orally to 3.2 ml, doses differing by a factor of 2. One group was retained as a control group receiving 3.2 ml of water orally; the remaining group was handled in the same manner as the test groups but was not injected. After the single dose, surviving animals were sacrificed 2, 5, 6, 7, 8, 9 and 12 days with 2 animals taken from each group at each time of sacrifice.

Spontaneous Deaths

All animals receiving 3.2 ml (21.8 g/kg) and 1.6 ml (10.9 g/kg) of DMMPA died within 2 hours of dosing. In addition, 2 animals receiving 0.8 ml (5.4 g/kg) died in 3 hours. Autopsy findings revealed severely congested lungs with haemorrhage into the alveolar space. Almost the entire lung was affected with 40% of the animals showing blood in the thoracic cavity. The livers of these animals were also severely congested. Kidneys of the highest dose group were congested with blood and oedematous. Histologically, they demonstrated glomerular and proximal tubular damage. Incidence of kidney damage of the animals dead from 10.9 g/kg DMMPA was 2/15. Neither of the animals dead from the 5.4 g/kg dose had damaged kidneys. All other tissues were normal to gross and histological examination in all dose groups.

Surviving Animals

All animals surviving DMMPA looked and acted normal within 2-3 hours after application although many demonstrated a high degree of agitation, sensitivity and mobility immediately after dosage. Findings on these animals will be reported under convenient headings although it is realized that many of the reported parameters are inter-related.

Food Intake

The food intake (Purina Rat Chow) per day is reported in Table IV. The values in the table represent the mean of measured food intake for the period ending on the day noted. Thus the value in day 5 represents the mean of intakes on days 1, 2, 3 and 4 for the animals sacrificed on day 5. This method of notation is used to assist comparison with other parameters which are measured only on the day of sacrifice. These data indicate that DMMPA even at a dose as high as 5.4 g/kg did not cause a significant change in food intake from the control animals who consumed 16.2 g/day (S.D. 5.3) of food.

Water Intake

Water intake, also measured daily, is reported in Table V following the same procedure used for display of food intake. These data show no significant change of water intake in animals dosed as high as 4.5 g/kg DMMPA from the control animals who showed water consumption of 27.2 ml (S.D. 4.7) per day.

Urine Analysis

Urines were collected daily from survivors and examined for the presence of blood, glucose, bilirubin, ketones and protein. The urines were all negative for the first four components. Control urines showed (as do all rats of this strain) a 1+ protein but this value was not increased in any of the urines from animals receiving DMMPA.

Respiration Rate

The rates of respiration in animals dosed with DMMPA were measured 2-3 times on each animal during the 12 days following dosing and a similar number of times in the control animals. The results are shown in Table VI. As can be seen in the table there is no significant difference in the breathing rates of any of the dosed animals over the 12 day recovery time in comparison with the control animals. However, the results on day 2 suggest

TABLE IV

FOOD INTAKE (GRAMS) VARIATION WITH DOSAGE OF DMMPA AND TIME AFTER DOSING

Dose		DAYS AFTER DOSE							mean of
ml	g/kg	2	5	6	7	8	9	12	dose group
0.1	0.9	14.3	14.3	13.0	14.8	14.3	16.3	17.2	15.0 (2.9)
0.2	1.8	16.0	17.0	14.8	14.8	16.5	16.3	15.1	16.5 (3.9)
0.4	2.7	14.8	13.3	14.3	15.0	13.8	16.5	15.2	14.8 (3.6)
0.8	5.4	13.8	11.7	11.3	15.8	16.5	16.5	18.0	14.8 (4.5)
Day Mean		14.7	13.4	13.4	15.1	15.3	16.4	16.6	
Control		12.5	15.5	15.5	13.3	13.5	16.3	21.0	16.2 (5.3)

TABLE V

WATER INTAKE

MILLILITERS OF WATER PER DAY IN RATS DOSED WITH DMPA

Dose Applied ml	TIME - DAYS AFTER DOSING							Mean for dose group		
	2	5	6	7	8	9	12	M	S.D.	N
0.1	24.0	25.0	22.2	29.3	32.5	27.0	26.4	27.4	5.5	44
0.2	27.4	24.2	29.5	27.8	32.3	26.5	24.6	27.3	4.8	38
0.4	22.6	23.2	26.8	28.8	32.6	28.2	26.2	27.5	6.3	46
0.8	24.2	24.8	24.7	27.6	32.2	28.6	26.0	27.1	7.4	38
Mean Per day	24.6	24.3	25.8	28.4	32.3	27.6	25.8			
Control	20.0	20.5	24.5	23.5	24.5	31.6	28.7	27.2	4.7	41

TABLE VI

RESPIRATION RATE

BREATHS PER MINUTE IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	155	178	179	183	176	187	187	176	18.6	30
0.2	1.4	149	156	186	193	195	195	185	176	24.4	20
0.4	2.7	152	167	203	170	190	200	195	188	13.7	25
0.8	5.4	156	137	145	196	185	186	183	165	28.1	24
Control		178	197	197	197	183	185	193	188	13.2	24

that immediately after the application of DMMPA there is a suppression of breathing rate. This is present at all dose levels from 0.1 to 0.8 ml per animal although the suppression is not apparently proportional to the dose applied. By day 5, the respiration rate has returned to normal. Since this result is the response of only 2 animals (4 observations) in each dose group, the statistical significance is not valid but will be examined in a later trial.

Heart Rate

Heart rate was taken 2-3 times on each test animal during the 12 days following DMMPA dosing and in the controls. The results are given in Table VII in which heart rates for successive periods are averaged and the mean heart rate for each dose group for the entire period recorded. There is no evidence of significant change in rate from the controls during the period of recovery, nor is the mean rate for the entire period significantly different from the control animals.

Body Temperature

Values of body temperature of the test and control animals are recorded in Table VIII. There is no evidence that DMMPA affects body temperature either early or later in the recovery from any dose of DMMPA.

Blood Pressure

Similar measurements of this physiological parameter are recorded in Table IX. Again there is no evidence that blood pressure is altered in the animals dosed with DMMPA either early or later in recovery period.

HAEMATOLOGICAL PARAMETERS

Total Erythrocytes

Changes in the total erythrocyte content of the blood as determined by an automatic cell counter are recorded in Table X, for animals dosed with DMMPA. With doses of DMMPA above 0.1 ml per animal there would appear to be

TABLE VII

HEART BEATS PER MINUTE IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	376	396	410	369	390	408	408	382	39.1	30
0.2	1.4	404	366	398	372	396	360	384	382	26.0	20
0.4	2.7	438	408	408	408	408	408	384	407	18.4	25
0.8	5.4	408	342	402	390	393	384	390	382	25.7	24
Control		396	402	390	353	390	372	358	379	23.6	24

TABLE VIII

BODY TEMPERATURE

DEGREES CELSIUS IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	*g per kg*	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	37.1	37.0	37.3	37.2	37.1	37.4	36.8	37.1	0.37	30
0.2	1.4	36.6	36.9	36.5	37.3	37.8	37.5	37.2	37.0	0.61	20
0.4	2.7	36.5	37.4	37.8	37.9	36.9	37.7	37.5	37.4	0.63	25
0.8	5.4	37.2	37.0	37.1	37.6	37.1	36.8	36.9	37.1	0.46	24
Control		36.7	36.9	37.9	36.4	36.3	36.6	36.7	36.8	0.59	14

TABLE IX

BLOOD PRESSURE

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	119	125	128	124	130	136	123	127	9.2	29
0.2	1.4	132	120	129	130	131	127	126	129	7.4	20
0.4	2.7	127	124	125	128	126	112	126	125	7.2	23
0.8	5.4	124	129	124	127	126	129	123	126	6.7	24
Control		126	126	132	117	125	119	128	126	8.8	24

TABLE X

RED BLOOD CELLS

RED BLOOD CELLS x 10^6 PER mm^3 IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	7.6	9.5	9.6	8.4	8.6	11.2	6.4	9.5	2.0	10
0.2	1.4	5.9	10.8	9.7	9.8	8.8	8.0	10.2	9.6	1.4	13
0.4	2.7	5.7	9.5	6.2	8.6	8.1	8.0	9.4	8.4	1.9	13
0.8	5.4	5.8	8.8	8.4	8.3	7.2	9.4	9.7	8.6	1.2	14
Control		7.7	9.2	9.3	7.6	8.0	8.1	8.9	8.4	0.7	14

a suppression of total RBC count up to day 2, although there is no significant change with any dose of DMMPA from day 5 to day 12, possibly indicating that the condition causing the cell count suppression in high doses underwent repair or recovery after 2 days. However, the validity of this observation is questionable since the observation was restricted to 2 animals at each dose of DMMPA used. This observation will be examined in a subsequent test.

Total Leucocytes

Change in total white blood cells is given over the same time span in Table XI. Again there is evidence of suppression of leucocyte count up to day 2, but no evidence of significant change in any time after that. There is also no significant change in the mean white blood cell count for each dose group over the entire recovery time examined in comparison to the control. Again the validity of the suppression of WBC up to day 2 of recovery is questionable and will be the subject of a later investigation.

Total Haemoglobin

Values of haemoglobin reported in Table XII follow the same pattern as the total erythrocyte count, as could be expected. As well there are low haemoglobin values up to day 2 of recovery with all doses of DMMPA above 0.1 ml per animal but no significant change is seen thereafter. Nor is there any significant alteration in total haemoglobin at any dose level of DMMPA when compared to controls for the entire period of recovery, indicating that the process of suppression seen in the first 2 days after exposure is one from which the animal recovers.

Haematocrit

The percent cell volume change with recovery time is shown in Table XIII. As with the other haematological parameters studied, there is evidence of questionable significance that the cell volume is suppressed in the first 2 days of recovery, but is entirely normal from day 5 onward.

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TABLE XI

WHITE BLOOD CELLS

WHITE BLOOD CELLS x 10^3 PER mm^3 IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	3.4	10.9	8.9	7.4	13.5	10.0	9.6	9.0	3.6	13
0.2	1.4	3.6	11.9	9.1	7.0	9.1	7.6	10.0	8.4	2.6	14
0.4	2.7	4.3	13.0	9.2	7.7	10.6	8.4	11.0	9.2	3.8	15
0.8	5.4	3.2	11.5	9.8	5.1	9.9	6.9	12.6	7.9	3.4	14
Control		7.2	10.2	7.9	9.5	7.9	10.0	8.9	8.8	2.4	14

TABLE XII

HAEMOGLOBIN

HAEMOGLOBIN IN GRAMS PER 100 ML BLOOD IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	12.1	18.1	15.0	14.3	---	15.4	12.0	15.3	2.6	9
0.2	1.4	10.3	18.6	15.7	16.5	---	14.3	17.5	16.9	2.0	11
0.4	2.7	10.8	16.8	12.6	14.8	---	14.9	17.6	15.3	2.4	11
0.8	5.4	10.8	16.9	15.8	13.8	---	14.1	16.3	15.5	1.4	9
Control		15.1	17.7	16.6	12.7	---	15.5	15.8	15.2	2.0	10

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TABLE XIII

HAEMATOCRIT

HAEMATOCRIT - % RBC VOLUME IN RATS DOSED WITH DMMPA

VARIATION WITH RECOVERY TIME

Dose Applied		TIME - DAYS AFTER DOSING							Mean for dose group		
ml per animal	g per kg	2	5	6	7	8	9	12	M	S.D.	N
0.1	0.7	34	44.5	41	40	48	46	43	44	4.3	10
0.2	1.4	24	44	40	48	47	44	44	45	6.4	13
0.4	2.7	28	42	36	46	44	45	46	42	6.3	13
0.8	5.4	24	41	44	41	42	45	47	44	4.9	11
Control		42	42	40	41	43	47	42	46	6.7	12

AUTOPSY RESULTS*

A summary of the pathological findings in rats treated with DMMPA and sacrificed is reported in Table XIV. There are no pathological findings which are related to the dose of DMMPA applied. Gross and histological examinations indicate random incidences of abnormalities which would be expected among groups of rats of the size used. However, even these findings are not totally acceptable inasmuch as the early responses to DMMPA leading to recognizable pathological lesions are detectable only in 2 animals per dose group (i.e. up to day 2). These findings are preliminary and will be subjected to further study, particularly on the short term responses in the subsequent study.

SECTION E

ACUTE DOSING OF RATS WITH DMMPA AND CHANGES IN THE FIRST 48 HOURS

Because of the results of the previous run suggesting a depression of respiration rate, a suppression of RBC, WBC, haemoglobin and haematocrit in the first 2 days after DMMPA, an experiment was designed in which matched weight groups of 12 rats each were exposed orally to 0.6 ml (35 g/kg), 0.8 ml (4.7 g/kg) and 1.0 ml (5.8 g/kg) and compared to a like control group receiving 1.0 ml of water orally. These animals were given pre-test examinations for heart rate, blood pressure, respiration rate and body temperature as well as recording of food, water intake and normal weight gain during two week pre-test period.

The animals were then dosed according to protocol and sacrificed by decapitation 12, 24, 36 and 48 hours later taking 3 animals at each time from each dose and control group.

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* Certain screening tests were not done in this experiment. Subsequent trials will include organ weights, water content of tissues and serum analysis.

TABLE XIV

SINGLE DOSE APPLICATION OF DMMPA TO RATS - SUMMARY OF AUTOPSY RESULTS

Dose Applied ml per animal	Dose Applied g per kg	Lung	Heart	Liver	Spleen	Kidney	Gut	Ovary	Bladder	Notes
0.1	0.7	N 14/15 A 1/15[a]	N 15/15	N 15/15	N 15/15	N 14/15 A 1/15[b]	N 15/15	N 15/15	N 15/15	All animals appeared normal grossly.
0.2	1.4	N 15/15	N 15/15	N 14/15 A 1/15[c]	N 15/15	N 15/15	N 15/15	N 15/15	N 15/15	As above
0.4	2.7	N 14/15 A 1/15[d]	N 15/15	N 14/15 A 1/15[e]	N 15/15	N 15/15	N 15/15	N 15/15	N 15/15	As above
0.8	5.4	N 12/13 A 1/13[f]	N 13/13	N 13/13	N 13/13	N 12/13 A 1/13[g]	N 10/13 A 3/13[h]	N 15/15	N 15/15	As above. NOTE: 2 animals which died within 3 hours of dosing are not included.
Control		N 13/14 A 1/14[j]	N 14/14	N 13/14 A 1/14[k]	N 14/14	N 14/14	N 14/14	N 14/14	N 14/14	One animal with axillary tumour and evidence of liver and renal damage removed from series. All other animals normal.

N - no evidence of pathological lesions.
A - Abnormal - pathological signs described in footnotes on following page.

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FOOTNOTES - AUTOPSY SUMMARY

(a) Animal sacrificed Day 12
Showed spotty lung which was composed of fibrous tissue likely the result of pre-test infection.

(b) Animal sacrificed Day 9
Kidney appeared normal grossly but sections showed some damage to kidney tubules.

(c) Animal sacrificed Day 7
Liver had small yellowish necrotic area in median lobe.

(d) Animal sacrificed Day 2
Fluid in thorax cavity, lung congestion with blood.

(e) Animal sacrificed Day 9
Liver normal size but spotted with white fibrous spots.

(f) Animal sacrificed Day 7
Lung showed moderate congestion but thorax contained excessive amounts of fluid somewhat blood stained. No infarcts were found.

(g) Animal sacrificed Day 12
Kidney was enlarged, spongy and possibly oedematous. Histological appearance normal.

(h) Animals sacrificed Days 5, 7 and 12.
All showed similar abnormalities of the ileal region in which some evidence of damage to the mucosal layer was present. Possible gastro-intestinal infection but not verified.

(j) Animal sacrificed Day 2
Lung was spottily haemorrhagic with fluid in thoracic cavity. Normal appearance in histological examination.

(k) Animal sacrificed Day 12
Liver was small, covered with a reticulated pattern of brown spots. No abnormalities seen histologically.

PHYSIOLOGICAL PARAMETERS

In Table XV the results of the measurement of rectal temperature, blood pressure, heart rate and respiration rate are recorded for each dose group and control for 12, 24, 36 and 48 hours after exposure orally to DMMPA. The values of the means of each parameter for 24 observations in each case before the administration of DMMPA are given in Column 3. No standard deviations are quoted since comparisons for significance of change after DMMPA were not made on these values, they are included for visual comparison only. The calculation of the significance of the difference was made only with the values in the control animals at the same period and under the same conditions. This procedure was also followed in testing the significance of the mean values of the dose group collectively and there comparison was made with the mean of the control animals considered collectively.

Rectal Temperature

From the results obtained there is no evidence of a significant change in rectal temperature in the first 48 hours after exposure to DMMPA.

Blood Pressure

There is a highly significant decrease in blood pressure in rats exposed to DMMPA which lasts throughout the 48 hour test period. The depression is the same for all doses of DMMPA used and thus, within the range of doses used, is not proportional to the dose of DMMPA applied.

Heart Rate

There is a highly significant decline in heart rate for all doses of DMMPA applied evident for the first 36 hours after dosing. By 48 hours this depression of heart rate has decreased to significant levels with 0.6 and 0.8 ml per animal dose but remains highly significant for the 1.0 ml dose. This change is likely evidence of recovery from the effect of DMMPA on this parameter in 48 hours.

TABLE XV

PHYSIOLOGICAL PARAMETERS IN RATS DOSED WITH DMMPA - VARIATION WITH RECOVERY TIME

Physiological Parameter	Dose Applied ml per animal	Prior to DMMPA Mean of N=24	Time - Hours After Dosing				Mean for Dose Group		
			12	24	36	48	M	S.D.	N
Rectal Temperature °C	0.6	37.04	37.42	37.40	36.87	36.87	37.13	0.54	24
	0.8	36.88	36.83	36.90	36.52	36.54	36.70	0.30	24
	1.0	36.92	37.36	37.29	36.86	36.62	37.0	0.53	24
	Control	36.94	36.77	36.76	36.67	36.74	36.7	0.08	24
Blood Pressure mm/Hg	0.6	126.6	117**	116.7**	118.3**	117.7**	117.5**	1.88	24
	0.8	126.0	118.7**	117**	119.7**	118.0**	118.3**	2.55	24
	1.0	125.1	112.7**	116.7**	118.3**	115.0**	115.6**	3.23	24
	Control	126.0	124.0	125.3	126.0	124.3	125.0	1.45	24
Heart Rate beats/min	0.6	341	304**	291.7**	304**	322.0*	305**	19.5	24
	0.8	347	306**	312**	329**	324*	314*	17.5	24
	1.0	349	300**	304**	328**	300**	308**	13.5	24
	Control	343	336	348	348	348	344.5	14.8	24
Respiration Rate breaths/min	0.6	172	140**	147**	155**	157**	150**	9.9	24
	0.8	173	153**	151*	164*	157**	156**	8.9	24
	1.0	173	137**	146**	158**	144**	146**	10.1	24
	Control	172	171	173	173	173	172**	3.3	24

Respiration Rate

There is a highly significant to significant decrease in breathing rate in the rats exposed to all doses of DMMPA which is evident for the first 48 hours after dosing with no evidence of recovery.

HAEMATOLOGY

Table XVI contains a summary of the RBC, WBC, haemoglobin and haematocrit values of rats exposed to DMMPA. There is no evidence in these data that there is any significant change in these parameters after DMMPA exposure. The previous observation indicating a possible decline in these parameters after DMMPA is not validated by this experiment. Since the previous observation had been made on only 2 animals at each dose, and the current experiment used 12 animals per dose, the conclusion that DMMPA does not affect haematological parameters will be based on this experiment.

Blood Serum Components

Table XVII summarizes the observations made on changes in blood serum components from rats exposed to DMMPA for a period up to 48 hours. There is no evidence among the 8 stable compounds of the rat blood serum or among the 4 enzyme components of any significant change occurring as the result of DMMPA dosing.

AUTOSPSY FINDINGS

All animals were autopsied within 6 hours of sacrifice in both the test and control groups. Cadavers were stored at +5°C until autopsy. Tissue weights were taken from the tissues immediately after removal and are given as a percentage of body weight. A sample of these tissues was set aside for determination of water content of the tissues. Gross findings were supported by histological examination of excised tissues after staining with haematoxylin and eosin.

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TABLE XVI

HAEMATOLOGICAL CHANGE IN RATS DOSED WITH DMMPA

Physiological Parameter	Dose Applied ml per animal	Time - Hours After Dosing				Mean for Dose Group		
		12	24	36	48	M	S.D.	N
R.B.C. x 10^6	0.6	7.15	7.01	7.63	6.82	7.15	0.41	12
	0.8	7.39	7.60	7.13	6.65	7.08	0.36	12
	1.0	7.31	7.05	7.20	6.82	7.35	0.84	12
	Control	7.06	7.10	6.58	7.09	6.96	0.49	12
W.B.C. x 10^3	0.6	4.85	8.77	6.85	7.83	6.95	1.98	12
	0.8	5.00	8.86	8.65	5.95	6.37	2.14	12
	1.0	5.88	9.26	5.77	6.41	6.83	2.06	12
	Control	5.29	7.88	4.40	8.2	6.44	2.55	12
Haemoglobin g /100 ml	0.6	15.8	17.5	18.1	15.0	16.6	1.33	12
	0.8	17.0	17.6	17.1	14.8	16.6	1.37	12
	1.0	16.7	17.4	18.0	15.1	16.8	1.27	12
	Control	16.3	16.7	16.3	15.2	16.1	1.13	12
Haematocrit	0.6	42.7	45.0	45.3	46.3	45.1	1.98	12
	0.8	46.3	47.7	46.7	45.0	46.7	1.23	12
	1.0	46.7	46.3	47.0	46.3	46.6	1.62	12
	Control	45.3	45.7	46.0	46.7	45.9	1.56	12

TABLE XVII

BLOOD SERUM COMPONENTS IN RATS EXPOSED TO SINGLE DOSE OF DMMPA

Serum Component	Dose Applied ml. per animal	Time - Hours After Dosing				Mean for Dose Group		
		12	24	36	48	M	S.D.	N
Sodium MEQ/l	0.6	155	158	158	153	155.8	4.52	12
	0.8	146	154	154	157	152.7	6.08	12
	1.0	153	155	154	148	152.3	4.81	12
	Control	151	154	156	149.4	152.8	5.88	12
Potassium MEQ/l	0.6	6.06	6.06	5.94	5.88	5.99	0.16	12
	0.8	5.76	6.00	5.82	5.94	5.90	0.20	12
	1.0	5.88	6.00	6.12	5.82	5.95	0.24	12
	Control	5.76	6.00	6.18	5.70	5.91	0.33	12
Blood Urea Nitrogen mg/100 ml	0.6	18.5	18.0	19.4	19.1	18.7	1.21	12
	0.8	18.8	20.1	18.4	20.2	19.4	1.27	11
	1.0	19.4	20.9	19.0	18.4	19.4	1.26	12
	Control	19.7	19.9	20.3	17.8	19.4	1.42	12
Blood Glucose mg/100 ml	0.6	129	151	140	147	141.9	26.4	12
	0.8	131	167	146	157	150.3	22.8	12
	1.0	140	132	128	130	132.4	13.4	12
	Control	125	139	136	123	130.6	16.8	12
Chloride MEQ/l	0.6	131.7	138.2	125.2	133.3	132.1	6.65	12
	0.8	135.	140.6	127.2	123.4	131.6	7.20	12
	1.0	131.3	138.0	130.5	121.5	130.3	7.51	12
	Control	136.3	139.7	136.6	120.7	133.3	8.06	12
Inorganic Phosphorus mg/100 ml	0.6	5.9	5.6	5.3	6.2	5.8	0.55	12
	0.8	6.1	5.7	5.7	6.1	5.9	0.31	12
	1.0	5.6	6.0	5.6	6.2	5.8	0.54	12
	Control	5.9	6.0	6.0	6.1	6.0	0.44	12

TABLE XVII

CONTINUED

Lactic Dehydrogenase	0.6	472	394	523	433	456	88.9	12
	0.8	537	315	528	479	463	98.0	12
	1.0	530	428	480	677	526	109	12
	Control	574	407	594	600	543	104	12
Alkaline Phosphatase	0.6	82	73	68	78	75	7.0	12
	0.8	81	72	72	63	72	10.0	12
	1.0	71	70	75	87	76	10.5	12
	Control	67	68	83	83	75	10.1	12
Creative Phosphokinase	0.6	138	151	143	135	142	12.9	12
	0.8	149	111	131	147	134	18.9	12
	1.0	152	136	123	159	142	18.8	12
	Control	136	125	134	152	136	20.0	12
Glutamic-Oxalic Transaminase	0.6	25.2	24.0	22.7	28.3	25.0	3.31	12
	0.8	20.8	19.3	21.1	27.3	22.1	3.60	12
	1.0	27.6	21.5	21.2	28.8	24.8	4.22	12
	Control	26.7	24.1	22.2	25.6	24.7	2.14	12
Total Protein g/100 ml	0.6	7.0	8.1	7.6	6.9	7.4	0.71	12
	0.8	6.4	8.7	7.8	6.2	7.3	1.13	12
	1.0	7.3	8.5	7.9	6.4	7.5	0.91	12
	Control	7.9	8.5	6.9	7.8	7.8	0.81	12
Albumin g/100 ml	0.6	4.4	5.2	5.2	4.5	4.8	0.70	12
	0.8	3.8	4.5	4.4	4.2	4.3	0.71	12
	1.0	4.0	4.1	4.9	3.9	4.2	0.67	12
	Control	4.7	5.2	4.5	5.3	5.0	1.00	12

Tissue Weights

The weights of 5 tissues as a percentage of body weight of the animal are shown in Table XVIII. There is no evidence indicating significant weight change in the lung, heart, liver, spleen and kidney in the animals exposed to DMMPA. These tissues have the same weight distribution as in the control animals.

Water Content of Tissues

The water content of the lung, heart, liver, spleen, kidney and skeletal muscle taken from animals exposed to DMMPA is shown in Table XIX and compared to the water content of the tissues from control animals. There is no evidence that DMMPA caused any significant change in the water content of the tissues in comparison to the controls.

Gross and Histological Findings

The autopsy findings are summarized in Table XX. These findings indicate that, within the dose range of DMMPA used and the time frame of the experiment, the only pathological findings are in the lung. The lung lesions are observable as early as 12 hours after dosing and appear to vary in severity as the dose of DMMPA is increased. The lungs seem to undergo a process of rapid recovery such that normal to only mild pathological signs are seen by 48 hours. There was evidence of a gastrointestinal disorder in the highest dose of DMMPA which was assigned to a gastroenteritis rather than a direct effect of DMMPA since it was seen in only 6/12 animals getting this dose.

TABLE XVIII

TISSUE WEIGHTS OF RATS AFTER ORAL EXPOSURE TO DMMPA

Tissue	Dose Applied ml. per animal	Time - Hours After Dosing				Mean for Dose Group		
		12	24	36	48	M	S.D.	N
Lung	0.6	0.43 *	0.43	0.50	0.45	0.45	0.041	12
	0.8	0.47	0.50	0.50	0.43	0.53	0.053	12
	1.0	0.51	0.50	0.61	0.47	0.523	0.064	12
	Control	0.57	0.57	0.50	0.48	0.53	0.053	12
Heart	0.6	0.321	0.34	0.29	0.34	0.32	0.018	12
	0.8	0.34	0.34	0.30	0.33	0.33	0.021	12
	1.0	0.35	0.36	0.34	0.29	0.33	0.031	12
	Control	0.35	0.36	0.30	0.31	0.33	0.028	12
Liver	0.6	3.0	3.6	3.0	3.9	3.42	0.47	12
	0.8	3.2	3.6	3.4	3.8	3.50	0.29	12
	1.0	3.4	3.2	3.3	4.0	3.49	0.42	12
	Control	3.3	3.8	3.0	3.4	3.28	0.51	12
Spleen	0.6	0.21	0.19	0.17	0.19	0.19	0.021	13
	0.8	0.18	0.19	0.18	0.19	0.19	0.015	12
	1.0	0.19	0.20	0.18	0.19	0.19	0.019	12
	Control	0.25	0.21	0.19	0.18	0.20	0.025	12
Kidney	0.6	0.39	0.38	0.36	0.40	0.38	0.029	12
	0.8	0.41	0.39	0.38	0.38	0.39	0.025	12
	1.0	0.42	0.40	0.40	0.40	0.40	0.031	12
	Control	0.36	0.41	0.37	0.38	0.38	0.036	12

* values shown are given as percent of body weight

TABLE XIX

WATER CONTENT - % OF WET WEIGHT OF TISSUES TAKEN FROM RATS EXPOSED TO DMMPA

Tissue	Dose Applied ml. per animal	Time - Hours After Dosing				Mean for Dose Group		
		12	24	36	48	M	S.D.	N
Lung	0.6	81	80.6	80	82	80.6	1.4	12
	0.8	81.7	81.0	83.0	81.0	81.8	1.4	12
	1.0	79.3	83.0	81.0	81.3	81.1	2.56	12
	Control	79.3	80.3	77.3	81.0	78.5	3.09	12
Heart	0.6	78	78.0	77	78.0	77.9	0.90	12
	0.8	77.3	78.0	78.3	78.3	78.0	1.05	12
	1.0	76.3	79.0	79.0	77.7	78.0	1.60	12
	Control	77.7	78.3	77.7	78.0	77.9	1.00	12
Liver	0.6	70.3	71.0	71.0	71	70.6*	0.79	12
	0.8	71.7	72.0	74.0	72.0	72.3*	1.78	12
	1.0	71.0	72.3	72.3	72.3	72.0*	1.41	12
	Control	70.0	70.0	72.7	70.7	71.0	1.28	12
Spleen	0.6	76	77.0	76	76.7	76.5	1.00	12
	0.8	78.3	77.0	78.3	77.0	77.7	2.50	12
	1.0	74.7	77.0	73.7	74.7	74.8	3.59	12
	Control	75.6	76.3	75.6	75.3	75.8	1.36	12
Kidney	0.6	75.3	78.3	75	77	76.5	3.37	12
	0.7	74.0	75.3	76.3	79.3	76.3	3.44	12
	1.0	71.7	77.0	74.7	77.3	75.3	3.36	12
	Control	72.7	78.7	73.7	72.0	74.3	4.81	12
Skeletal Muscle	0.6	77	75.3	76	77.3	76.3	1.88	12
	0.8	75.7	74.7	77.6	78.7	76.6	2.71	12
	1.0	76.6	77.7	75.3	77.0	76.6	1.06	12
	Control	77.3	77.0	78.0	77.3	77.4	1.31	12

* Significantly different from control.

TABLE XX

SUMMARY OF AUTOPSY FINDINGS IN RATS DOSED WITH DMMPA

Dose DMMPA		12 hours	24 hours	36 hours	48 hours
ml per animal	g per kg				
0.6	3.5	Haemorrhagic spots on the lung in 3/3 animals. All other tissues normal.	Normal lungs 2/3. Slight haemorrhagic areas 1/3. All other tissues normal.	Mild congestion and oedema in lungs 3/3 animals. All other tissues normal.	All tissues normal.
0.8	4.7	Mild to moderate haemorrhagic spots in lungs of 3/3 animals. All other tissues normal.	Oedema and congestion in lungs 3/3 animals. All other tissues normal.	Areas of haemorrhage and congestion in lungs of 2/3 animals. All other tissues normal.	Mild petecheal haemorrhage areas in lungs 3/3 animals. All other tissues normal.
1.0	5.8	Oedema, congestion and haemorrhage in lungs of 3/3 animals.	Spotty to dense haemorrhagic areas with congestion and oedema in lungs of 3/3 animals. Gut wall degeneration from gastroenteritis in 2/3 animals.	Heavy congestion with oedema in lung 3/3 animals. Gut wall degeneration in 3/3 animals, likely the result of gastroenteritis.	Mild to moderate congestion in lungs of 3/3 animals.
Control	—	Normal	Normal	Normal	Normal

DISCUSSION

Assessment of the safety of a compound is customarily done through the calculation of the ratio between the dose anticipated in use and the minimum dose found to produce pathological effects. Usually the latter dose is estimated from the maximal dose producing no pathological effects.

One estimate of the maximum no-effect dose can be made from the data used to develop the LD_{50} estimates of lethality. If the oral route is chosen, the LD_{50} value of DMMPA in rats (male) is 5.9 g/kg. The LD_1, i.e. the dose lethal to 1% of the animals, was 3.41 g/kg with the 95% fiducial limits 2.29 to 4.04 g/kg. If the lower end of these fiducial limits is assumed to approach the LD_0, then 2.3 g/kg can be taken as the highest dose likely to cause no deaths. From a practical point of view, in the some 200 animals studied, no deaths occurred at this level of acute dosage.

Other estimates of the no-effect dose can be made from the various body functions or parameters screened. A summary of such doses is made in Table XXI. As can be seen, if a 5 day recovery period is used, which means that any lesion produced will undergo repair in this time frame, the maximum no-effect dose is 5.4 g/kg. This level, for some entities, is reduced to lower doses when a 2 day only recovery period is permitted, but the likely overall no-effect estimate is still near the LD_0 dose of 2.3 g/kg. Inasmuch as many of the screening criteria used are considered more sensitive indexes of body change than lethality, the failure of these indexes to delineate doses below the LD_0 indicates either that such criteria are overrated or that DMMPA is a compound with the rare property of being free of long duration pathological effects. This, of course, allows the conclusion that DMMPA is a very safe compound for single dose acute use.

TABLE XXI

SUMMARY TABLE ON THE NO-EFFECT ACUTE DOSES OF DMMPA

Function Examined	Maximum Dose Having No Effect g/kg	
	Recover Period 2 days	Recovery Period 5 days
Food Intake	ND	5.4
Water Intake	ND	5.4
Urine Components	ND	5.4
Respiration Rate	<3.5	5.4
Heart Rate	<3.5	5.4
Blood Pressure	<3.5	5.4
Body Temperature	5.8	5.4
Total Erythrocyte Content	5.8	5.4
Total Leucocyte Content	5.8	5.4
Haemoglobin	5.8	5.4
Haematocrit	5.8	5.4
Blood Serum Components including enzymes 14 entities	5.8	5.4
Tissue Weights	5.8	5.4
Lung Damage	4.7	5.4

ND=Not Done

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ANNEX A

GENERAL METHODOLOGY

Animals held for periods longer than 24 hours were individually caged in round metabolism cages in which food and water intake and urine collection could be made. Animals were housed in two rooms, one holding 90 cages, the other 60 cages, with temperature maintained at 70 ± 2°F. Cages were changed weekly with clean cages which had been washed and sterilized before use.

Since the facility accommodated 150 rats, 8 groups of 15 test animals each were used with 2 groups retained for controls. Since all doses were given orally, one control group received water by the ball-point needle used for stomach dosing, while the other control group was handled but not injected. In all tests, the 8 test and 2 control groups were started with equal weight distribution in each group; i.e. there was no significant difference in the mean weights of each group at the beginning of an experimental run.

To reduce the risk of secondary infection, all animal personnel were required to wear special clothing in the animal rooms and to thoroughly scrub if they had been in contact with other laboratory animals before coming into contact with the toxic rats. Access to the toxicology rooms was restricted to those directly involved in the care, dosing and observation of the animals. Normal daylight and darkness periods were used, although since one room was windowless, artificial lights were on in this room to match daylight periods. However, air circulation in the rooms was not separate from the rest of the animal building and although air (65% interior and 35% exterior mixture) was returned through filters, there still existed the possibility of air infection. This occurred in one run only and the findings on this run were discarded completely.

Animals were sacrificed at fixed periods by decapitation to facilitate the collection of blood for haematology and biochemical analysis. Initially the animals were anaesthetized using nitrogen or carbon dioxide, but both methods were abandoned when it was found the methods caused certain degrees of lung damage.

All animals carried individual identification and were dealt with individually including individual case histories of all data collected on them. Efforts were made to disturb the animals as little as possible, and consequently measurements such as weight, physiological parameters, etc. were arranged to be done from mobile carts which moved to the cages. Animal personnel were encouraged to keep all noise to a minimum.

Records were kept of individual animal characteristics including behavioural change (if any). To facilitate after-hour observation of behaviour, one toxicology room was equipped with a low light level illumination during silent hours and the animals monitored with a low light level television camera. This system, although used in the initial long term chronic dosing trials, was later dropped since DMMPA produced little evidence by this (and other) means of behavioural change.

NUTRITION METHODS

Rats were supplied Purina Rat Chow ground to a coarse granular size of about 2 mm in diameter. Food was given in food cups holding about 50 grams each. The food was accessible to the animals through a perforated disc placed on top of the supply. The entire cup was closed with a cover containing a hole about 1 inch in diameter. To avoid spilling the cup was held with a metal spring. Daily food intake was recorded as the difference between the weight of the food and cup assembly before and after 24 hours feeding. No allowance was made for spillage, but this was very low.

Fresh water was supplied daily. Water intake was read from graduated water bottles attached to the side of the cages with metal springs. No urine preservative was used. Normal volumes of urine were not recorded since no attempt was made to estimate evaporation loss, however a record was kept of those animals demonstrating high or low urine excretion. Since analysis was made daily, urine collection was made on a 24 hour basis.

Animals were weighed daily to the nearest gram. For short term experiments, weight trends were calculated daily, but for long term experiments a mean value for 5 days was used to simplify calculations.

PHYSIOLOGICAL METHODS

All physiological methods were designed to be applied to normal unanaesthetized rats with minimum restraint. All are non-penetrative and as such may be used to give repetitive values on the same animal. All equipment necessary for the measurement of the four parameters was assembled on one portable cart which could be moved to the animal under study. The apparatus was described in detail in (5).

Respiration Rate

This apparatus consisted of a restraint tube holding the entire animal such that its head was contained in a dome at one end. The head of the animal was isolated by an inflatable cuff around the neck such that air could be breathed through a tube into the dome. Air movement, both rate and volume, were measured by the degree of temperature changes undergone by thermistors mounted in the air stream. Both volume and breath rate could be measured, although only the latter was determined in the studies reported here. The apparatus was calibrated for direct read-out of breath rate.

Heart Rate

Heart rate was measured by recording the pressure pulses detected in the tail via an inflatable cuff. A Statham pressure transducer was used to detect pressure pulses with this signal converted for direct read-out of heart rate.

Blood Pressure

This determination utilized the heart rate meter described above. A second pressure cuff connected to a pressure gauge reading in millimeters of mercury was applied to the tail proximal to the heart rate cuff. For determination, the pressure cuff was inflated until the pulse wave as observed on an oscilloscope became flat. The pressure was then slowly released and allowed to fall. The pressure at which the pulse wave first became visible on the oscilloscope was then read. The procedure was then repeated and the average of 3 readings recorded. The arterial pressure measured is likely close to the systolic pressure.

Body Temperature

This parameter was measured with a Yellow Springs rectal temperature probe and direct read out. Since the probe is a rapid responding one, the time necessary to leave the probe in the rectum was about 5 seconds. Paw temperature (although not needed after preliminary trials) can be measured simultaneously using a flat probe on which the animal's paw is pressed.

Allowing time for the animal to become adapted to the measurements, about 10-20 minutes is required for all physiological parameters to be determined in each animal.

Urine Analysis Methods

Qualitative tests for the presence of urine components was performed using Ames Bili-Labstix*. Protein, glucose, ketones, bilirubin and blood were tested with urine pH.

Haematology Methods - Blood Specimens and Sampling

Erythrocytes and leucocytes were counted after dilution in a Fisher Autocytometer II giving total counts of each cell type following the instructions supplied with this instrument. Total haemoglobin was determined colorimetrically simultaneously on the lysed specimen by an oxyhaemoglobin procedure. Haematocrit value was determined by capillary tube procedure after centrifuging at 8,000 rpm for 2 minutes.

Biochemical Methods

All analyses of chemical components and enzymes are determined on the Beckman Discrete Sample Analyser equipped with a flame photometer for sodium and potassium estimations. All methods used are standard clinical chemistry analytical procedures adapted for use in the Beckman DSA and supplied in a manual of operation methods for this instrument. Analysis was performed in batches of 10 serum samples, each batch preceded by a suitable standard serum sample. Analysis of fugitive components such as the enzymes was performed on the same day as the samples were collected. Stable components were analysed as convenient with storage of the serum at 5°C.

* Ames Co. Division of Miles Laboratory Ltd. Rexdale, Ontario, Canada

Autopsies

All animals in the tests were autopsied with records maintained by dictation into a tape recorder and later transferred to a standardized autopsy record form. Initially some 20 tissues were taken for organ weight and histological examination, but as the experiment proceeded this test was reduced to lung, liver, kidney, spleen and heart for organ weights and the same tissues with a sample of skeletal muscle for determination of water content. For the latter use, a small sample of blotted wet tissue was weighed on a planchette and dried to constant weight in a ventilated oven at 75°C. Tissues for histological examination included those mentioned above plus any tissues demonstrating gross abnormality. They were preserved in buffered formalin and stained with haematoxylin and eosin before cutting and mounting.

Scheme Used for Estimation of Severity of Eye Damage

The following scoring was used to estimate the degree of damage incurred in rabbit eyes after instillation of DMMPA.

1) General Impression of Eye (Score 0-5) A first look judgement on how the eye appears, where normal = 0 and a closed sensitive eye scales 5.

2) Presence of Exudate

Tears (Score 0-5) Level is based on presence of tears, wet fur around eyes.

Serum or Pus (Score 0-5) Level is based on amount of serum or pus present, including whether the eye is sealed shut by such exudates.

3) External Eye (Score 0-5) Presence of erythema, oedema and possibly some external eye lid erosion; degree of damage determines score.

4) Nictitating Membrane (Score 0-5) Presence of swelling, erythema, erosion and slough determines level of score.

5) Conjunctiva (Score 0-10) Presence of erythema, vascularity, slough and area of damage determines score.

6) Sclera (Score 0-5) Presence of erythema, slough, erosion and area of damage determines score.

7) Cornea - examination by slit lamp.

Presence of Pitting (Score 0-5)
Presence of Opacity (Score 0-10)
Presence of Slough (Score 0-5)
Presence of Vascularity (Score 0-5)

8) Iris (Score 0-5) Examination by slit lamp for evidence of degree of damage. Iris response to light as a round uniform aperture also contributes to score.

9) Vision (Score 0-2) Animal response to small object brought close to eye as blinking or withdrawal. Failure gives a score of 2.

Total damage is scored as sum of individual elements. Normal animals have a score from 0 to 5.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1 ORIGINATING ACTIVITY DEFENCE RESEARCH ESTABLISHMENT SUFFIELD	2a. DOCUMENT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP

3. DOCUMENT TITLE

TOXOCOLOGY OF DMMPA
PART I
THE ACUTE EFFECTS OF DMMPA

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)
TECHNICAL PAPER

5. AUTHOR(S) (Last name, first name, middle initial)

Coleman, I.W.

6. DOCUMENT DATE August 1977	7a. TOTAL NO. OF PAGES 52	7b. NO. OF REFS 7
8a. PROJECT OR GRANT NO. Project No. 13E11	9a. ORIGINATOR'S DOCUMENT NUMBER(S) SUFFIELD TECHNICAL PAPER NO. 466	
8b. CONTRACT NO.	9b. OTHER DOCUMENT NO.(S) (Any other numbers that may be assigned this document)	

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The toxicological properties of dimethyl morpholinophosphoramidate (DMMPA) have been examined in mice, rats and rabbits using a variety of criteria of toxicological responses. Nutritional, physiological, haematological and blood serum components were studied after DMMPA acute dosing. Macroscopic and microscopic pathology was also carried out. The maximum dose causing no pathological effects was in no case less than the LD_0 dose estimate, indicating the compound was remarkably free of pathological effect, and likely indicating the compound to be safe for acute exposures to small amounts to man.

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DSIS
76-476

KEY WORDS

Toxicology

DMMPA

Acute

Rats

Rabbits

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